遗传 2007 29 (3): 306—312 ISSN: 0379-4172 CN: 11-5450/R

研究报告

应用变性高效液相色谱技术建立胸苷酸合成酶基因多态检测平台

张晓梅，陈森清，曹海霞，吴建中，马国建，李金田

江苏省肿瘤防治研究所, 南京 210009

收稿日期 2006-4-3 修回日期 2006-12-26 网络版发布日期 2007-3-1 接受日期

摘要

*TS*基因5′非翻译区(5′ untranslation region, 5′ UTR)增强子区域(*TS* enhancer region, TSER)存在28 bp的2次(2R)、3次(3R)的串联重复多态，在3R等位基因第二次重复中还存在一个G→C的单核苷酸多态性(single nucleotide polymorphisms, SNPs)，同时在3′非翻译区(3′ untranslation region, 3′ UTR)存在6个碱基片段缺失/插入多态。这些多态形式的存在影响了*TS*基因mRNA的稳定和翻译效率，并可导致不同*TS*基因型肿瘤患者对以5-fuorouracil (5-FU)为基础的化疗疗效产生差异。为提高*TS*基因型临床检测的效率和准确性，方便、快捷、准确和自动化区分各种纯合及杂合基因型，设计多重PCR反应，同时扩增*TS*基因5′和3′非翻译区多态所处片段。利用DHPLC技术建立*TS*基因多态性检测平台，在非变性条件下，通过优化DHPLC 洗脱梯度，同时检测5′ TSER区的串联重复多态和3′ UTR片段长度多态；在变性条件下，检测5′ TSER区单核苷酸多态。同时采用PCR-RFLP和DNA 测序方法，验证DHPLC分析结果。

关键词 胸苷酸合成酶 基因多态性检测

分类号

Simultaneous detection of polymorphisms in the 3′- and 5′-UTR of thymidylate synthase gene using denaturing high-performance liquid chromatography

ZHANG Xiao-Mei, CHEN Sen-Qing, CAO Hai-Xia, WU Jian-Zhong, MA Guo-Jian, LI Jin-Tian

Jiangsu Institute of Cancer Research, Nanjing 210009, China

Abstract

<P>Polymorphism which is either in the thymidylate synthase (<EM>TS</EM>) enhancer region (TSER) of the 5-primer untranslation region (5′ UTR) or in the 3-primer untranslation region (3′ UTR) has been reported to be associated with the alterations in <EM>TS</EM> mRNA and protein levels. The <EM>TS</EM>ER is characteristic of the presence of variable double (2R) and triple (3R) number tandem repeats (VNTRs). In addition to VNTRs, single nucleotide polymorphism (SNPs) in 3R, as well as the polymorphism of the fragment length(FLP) in the <EM>TS</EM> 3′-UTR which is characteristic of the presence or absence of a 6 bp-nucleotide fragment, has recently been reported to be associated with the response to 5-fuorouracil (5-FU)-based chemo-therapy. The aim of the present study was to develop a specifc denaturing high-performance liquid chromatography (DHPLC) method for the rapid and simultaneous detection of these variations in clinical samples. Multi-PCR primers were designed to amplify the two regions simultaneously, The 8.6 min DHPLC gradient was optimized to include the analysis of multiplexed <EM>TS</EM>ER/3′ UTR chromatogram peaks, allowing for the simultaneous detection of 28 bp VNTRs and 6 bp FLP

under nondenaturing conditions (50℃). The optimal melting temperature was determined experimentally for the detection of SNP in the TSER VNTRs. Finally, the DHPLC analysis was verified in parallel with PCR-RFLP and sequencing. The optimized DHPLC method resolved 100% of the known <EM>TS</EM> variations, discriminated between homozygous and heterozy-gous genotypes. This developed DHPLC method could permit the rapid, sensitive, and accurate identification of the <EM>TS</EM> genotypes (VNTRs, SNPs, and FLP).
</P>

Key words DHPLC thymidylate synthase genetic polymorphism detection

DOI: 10.1360/yc-007-0306

通讯作者 张晓梅 zxm901·yahoo.com.cn